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Efficacy of amitraz applied as a dip against an amitraz-resistant strain of *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) infested on cattle☆

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Abstract

Selection pressure with the acaricide, amitraz was applied to a strain of *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) in 13 out of 18 generations, resulting in a 28.7-fold increase in resistance, indicating a shift in the phenotypic composition of the ticks, from a heterogenous mixture of both susceptible and resistant ticks when selection began to a homogenous composition of predominantly resistant ticks when selection ended. Resistant larvae of the last selected generation were infested on cattle three separate times at 20, 13, and 6 d (7 d intervals) before being dipped in a total immersion vat charged at 0.02, 0.044, and 0.096% active ingredient (AI) amitraz. There was a dose related, though not always significant, response to amitraz whereby each increase in concentration produced increased adverse effects. The number of ticks and index of fecundity (IF) of females recovered from cattle treated at 0.02 and 0.044% AI were not different from that of untreated ticks. However, female weight and egg mass weight of females recovered from cattle treated at 0.044% were lower than untreated females or females treated at 0.02% AI. All measured parameters obtained from ticks recovered from cattle treated at 0.096% AI were significantly lower than the untreated control group, but tick numbers and IF were not different from the other treated groups. At 0.02% AI the level of control was significantly lower (34.6%) than treatment at 0.096% AI (81.2%), while the level of control at 0.044% AI (57.6%) was intermediate between the other treated groups. Therefore, none of the amitraz treatments provided the required 99% control necessary for use in the U.S. Cattle Fever Tick Eradication Program, and the estimated line derived from regression analysis showed that a concentration of ca. 0.2% AI amitraz would be needed to achieve 99% control of the resistant ticks.
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Keywords: *Rhipicephalis* (*Boophilus*) *microplus*; Amitraz; Acaricide resistance; Formamidine

1. Introduction

The United States Cattle Fever Tick Eradication Program (CFTEP) carried out by the Veterinary Services (VS) branch of the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) has been in continuous operation for 100 years, since its inception in 1907. As a result of the CFTEP *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) and *R.* (*B.*) *annulatus* (Say) were eradicated

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from an area of 1,813,000 km^2 during the twentieth century (Graham and Hourrigan, 1977), along with the disease, bovine babesiosis for which these tick species are the sole vectors (Smith and Kilborne, 1893). Today intermittent incursions of *Rhipicephalus* (*Boophilus*) ticks still occur within the eight southern-most Texas counties that lie adjacent to the U.S.–Mexico border, where the program maintains a permanent quarantine zone to prevent the re-establishment of these ticks. One of the most challenging issues the CFTEP has ever faced has occurred in the past 25 years with the emergence and development of pesticide resistance to the principal acaricides that have been used to control these arthropod disease vectors.

Because of the large expanse of contiguous border between the U.S. and Mexico, the success of the U.S. CFTEP has always been inexorably linked to control efforts conducted in Mexico, where these ticks remain endemic. Since the initiation of the U.S. program, one of the main focal points of the campaign has been the systematic treatment of livestock with chemical acaricides. Therefore, the development of acaricide resistance in Mexican tick populations has been a grave concern to the U.S. program. While the CFTEP has relied almost exclusively on the use of the organophosphate (OP) acaricide, coumaphos as the only approved acaricide for treatment of cattle in eliminating *Rhipicephalus* (*Boophilus*) ticks during the last 40 years (Graham and Hourrigan, 1977), the extensive use of OP acaricides, as well as other classes of chemical agents, in Mexico has resulted in resistance to several major classes of chemicals. In Mexico, OP resistance developed in the mid 1980s and subsequently became widespread throughout the country, following which a switch to the intensive use of pyrethroid (P) acaricides resulted in high level resistance to this class of agents in the early 1990s (Aguirre et al., 1986; Santamaria and Fragoso, 1994; Fragoso et al., 1995; Santamaria et al., 1999). The development of OP and P resistance in Mexico led the U.S. eradication program to intensify efforts to find an alternative acaricide to the use of coumaphos that could potentially be used in the treatment of cattle at import facilities and on infested premises within the U.S. to prevent incursion by OP- and P-resistant tick populations.

One class of chemicals, the formamidines, of which amitraz is a member, has been of interest to the U.S. program for a number of years as a potential alternative to coumaphos. Previous investigations conducted in the U.S. against acaricide-susceptible *Rhipicephalus* (*Boophilus*) ticks showed that amitraz was highly effective (>98% control) at low concentrations (Davey et al., 1984; Ahrens et al., 1989; George et al., 1998). Amitraz was first introduced in Mexico to control OP-resistant ticks in 1986 (Aguirre et al., 1986; Soberanes et al., 2002) and it was adopted as the recommended acaricide treatment for OP- and P-resistant ticks in the 1990s when resistance to these chemicals became widespread (Parrodi et al., 1995). However, the first detection of amitraz resistance in a Mexican strain of *R.* (*B.*) *microplus* reported in 2001 (Soberanes et al., 2002) coupled with more recent findings showing low-order amitraz resistance in 11 Mexican tick strains collected from several major cattle-producing areas (Li et al., 2004) has caused the U.S. CFTEP to reconsider the potential use of amitraz in the U.S. program.

The purpose of this study was to determine the acute efficacy of amitraz against an amitraz-resistant strain of *R.* (*B.*) *microplus* using different concentrations of amitraz. Results of the study will provide critical information on the efficacy of amitraz applied at recommended concentrations against resistant ticks. Furthermore, results will provide insight into whether higher concentrations, applied against amitraz-resistant ticks, will provide the requisite 99% control that is necessary to obtain eradication of ticks detected on cattle presented for importation from Mexico or on cattle held and systematically treated on quarantined premises within the U.S.

2. Materials and methods

2.1. Tick strains

The origin of the amitraz-resistant tick strain used in the study was from a ranch located near the town of Emiliano Zapata, Tabasco, Mexico in 2001. Engorged females were collected and colonized for six generations at the National Center of Parasitology Laboratory, Jiutepec, Morales, Mexico, prior to being transferred to the USDA, Agricultural Research Service (ARS), Cattle Fever Tick Research Laboratory (CFTRL), Edinburg, TX in 2002. Therefore, the F_6 larval ticks that were obtained were designated as the F_6 generation at the beginning of the colonization process at the CFTRL, thus the first full generation of ticks established at the CFTRL was the F_7 generation. From the first generation of colonization (F_7) until the study was initiated (F_{25}) (a total of 18 generations) the strain was selectively pressured with amitraz in 13 of the 18 generations to maintain or increase the level of resistance in the strain. Amitraz concentrations used to selectively pressure the strain at each generation were always equal to or above the discriminating dose of 0.03% AI established by